EFFECT OF EXTRACTION CONDITIONS ON PROTEIN CONCENTRATION DURING EDIBLE BIRD'S NEST ALKALINE HYDROLYSIS

NURUL AIN BINTI JUMRI

Thesis submitted in partial fulfilment of the requirements
for the award of the degree of
Bachelor of Chemical Engineering (Biotechnology)

Faculty of Chemical & Natural Resources Engineering
UNIVERSITI MALAYSIA PAHANG

JUNE 2014

ABSTRACT

Edible bird's nest (EBN) is originated from the saliva of swiftlet. There are four species of swiftlet which are *Collocalia fuciphaga*, *Collocalia germanis*, *Collocalia maxima* and *Collocalia unicolor*. *Collocalia fuciphaga* is the common species which can be found in Malaysia. EBN consists mainly of amino acids, carbohydrates and mineral salts but it major ingredients were protein. Protein from EBN can be extracted by water, acid, alkaline and enzymatic hydrolysis method. This study focused on alkaline hydrolysis using sodium hydroxide (NaOH) at 50°C. Prior to alkaline hydrolysis, pre-treatment method (EBN cleaning by soaked with water, feathers removal and fine plumage from EBN sample) has been carried out. The effect of extraction time, NaOH concentration and liquid solid ratio (LSR) on protein concentration have been determined during alkaline hydrolysis. The optimum condition of alkaline hydrolysis has been identified using response surface methodology (RSM). From the experimental result, it showed that the maximum protein concentration was at 25 minutes of extraction time, 30:1 of LSR and 100 g/L NaOH concentration. However, increased in LSR and NaOH concentration were increased the protein concentration. The optimum condition of protein concentration was 1.260 g/L during alkaline hydrolysis using RSM were found at 25 minutes of extraction time, 100g/L NaOH concentration and 30:1 of LSR. From the ANOVA result, the significant factor was extraction time ($p=0.0187$) and was clearly showed that further increased in extraction time (25 minutes) resulted in decreased in protein concentration due to the degradation of amino acid structure at long duration of extraction time. The FTIR spectrums of the untreated and treated sample resulted in the different trend of spectrum. In the treated sample after pre-treatment process and alkaline hydrolysis, it is showed that N-H and C=O stretching peak is increased. Besides, it was confirmed that the material was correspond to amides as it consists of C=O stretch. While, from the analysis using the ICP-MS, amount of cadmium, arsenic and lead in the sample was 0.002mg/L, 0.00006mg/L and 0.262mg/L respectively after pre-treatment. It also showed that percentage decreasing of argentums, cadmium and magnesium is the highest compared to others metal which were 99%, 97.70% and 97.24% respectively. It can be concluded that EBN can be performed as an alternative source for protein.

ABSTRAK

Sarang burung berasal dari air liur burung walit. Terdapat empat spesies burung iaitu *Collocalia fuciphaga, Collocalia germanis, Collocalia maxima dan Collocalia unicolor*. *Collocalia fuciphaga* adalah spesies yang biasa didapati di Malaysia. EBN terdiri daripada asid amino, karbohidrat dan garam mineral tetapi bahan utamanya adalah protin. Protin dari EBN boleh diekstrak dengan air, asid, alkali dan kaedah enzimatik hidrolisis. Kajian ini tertumpu kepada hidrolisis alkali menggunakan natrium hidroksida (NaOH) pada 50°C. Sebelum hidrolisis alkali, kaedah rawatan awal (pembersihan EBN dengan merendam sampel dengan air, penyingkiran bulu kasar dan bulu halus dari sampel EBN) telah dijalankan. Kesan masa pengekstrakan, kepekatan NaOH dan nisbah pepejal cecair (LSR) kepada kepekatan protein telah ditentukan semasa proses hidrolisis alkali. Keadaan optimum hidrolisis alkali telah dikenal pasti menggunakan balas metodologi permukaan (RSM). Dari keputusan eksperimen, ia menunjukkan bahawa kepekatan protein maksimum dicapai pada 25 minit masa pengekstrakan, 30:1 nisbah pepejal kepada air (LSR) dan 100g/L kepekatan NaOH. Peningkatan dalam LSR dan kepekatan NaOH telah menyumbang kepada peningkatan kepekatan protein. Keadaan optimum kepekatan protein adalah 1.260g/L semasa hidrolisis alkali menggunakan RSM ditemui pada 25 minit masa pengekstrakan, 100g/L kepekatan NaOH dan nisbah 30:1. Dari keputusan ANOVA, faktor penting didapati adalah masa pengekstrakan ($p = 0.0187$) dan telah jelas menunjukkan bahawa peningkatan terus menerus pada masa pengekstrakan (25 minit) menyebabkan penurunan kepada kepekatan protein disebabkan oleh kemusnahan struktur asid amino oleh signal masa pengekstrakan. Spektrum FTIR sampel yang tidak dirawat dan dirawat menunjukkan trend spektrum yang berbeza. Dalam sampel yang dirawat selepas proses rawatan awal dan hidrolisis alkali, ia menunjukkan bahawa N-H dan C=O regangan puncak bertambah. Selain itu, ia telah mengesahkan bahawa bahan tersebut dikategorikan sebagai amida kerana terdiri daripada C =O regangan. Selain itu, dari analisis ICP-MS, jumlah kadmium, arsenik dan plumbum dalam sampel adalah 0.002mg/L, 0.00006mg/L dan 0.262mg/L masing-masing selepas rawatan awal. Ia juga menunjukkan bahawa peratusan penurunan argentums, kadmium dan magnesium adalah yang tertinggi berbanding dengan logam lain yang masing-masing 99%, 97.70% dan 97.24%. Ia boleh membuat kesimpulan bahawa EBN sesuai digunakan sebagai sumber alternatif untuk protein.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

Ppb	parts per billion
Ppm	parts per million

LIST OF ABBREVIATIONS

EBN	Edible bird's nest
FTIR	Fourier transform infrared spectroscopy
ICP-MS	Inductively coupled plasma-mass spectrometry
LSR	Liquid solid ratio
NaCI	Sodium chloride
NaOH	Sodium hydroxide
RSM	Response surface methodology

1 INTRODUCTION

1.1 Background of the study

Protein is a complex macromolecules made up of amino acids which are covalently bonded together through substituted amide linkages called peptide bonds (Rosenberg, 2005). The bonded is in head-to-tail arrangement. Each protein molecule is arranged in a linear that is unbranched fashion. Protein also known as the combination of amino acids in the peptide linkage containing carbon, hydrogen, oxygen, nitrogen and sulfur (Yada, 2004). There are some unfavoured conditions that tend to made protein molecules become unfolded and fully denatured. The unfavoured conditions are high temperature, an acidified condition, excessive shear and high pressure. Instead of being aggregated and/or crosslink to form larger cluster, denatured protein will form a three-dimensional solid-like network (or gel) at high concentration. Besides, protein is also be known as main classes of building blocks used in semi-solid food that acts for conferring mechanical properties (Linqiang *et al*., 2008)

Ebru *et al.*, (2010) has said that the standard used in order to quantify the amount of protein is bovine serum albumin (BSA). The definition of protein yield and extraction rate that assessed extraction method is the percentage ratio of the protein quantity extracted to the total amount of the protein. Method that is widely used to determine the protein content is known as The Lowry method. A modified cupric sulphate-tartrate reagent has been develop by Pierce,1996 (from the instruction manual) that acted to places two of the three reagents in the original established Lowry method with one stable reagent. This is due to the interest in avoiding the necessity to prepare the fresh reagent daily. About 100% correlation of the colour response curves with various proteins has been observed between the Pierce modified Lowry protein assay reagent and the original Lowry method.

Apart from that, there are three general methods that were widely used to hydrolyze protein into its composition which is amino acids. There are acid hydrolysis, alkaline hydrolysis and enzymatic hydrolysis. This study used alkaline hydrolysis based on its advantages. Among the advantages of alkaline hydrolysis included the time taken to complete hydrolysis. Relatively long periods required for the complete hydrolysis of bonds using acid hydrolysis. Besides, alkaline hydrolysis is more simples compared to enzymatic hydrolysis. This is because, most enzymes attack only specific peptide bonds rapidly which difficult the whole process. One of the important groups of emulsifying agents used in the food industry is protein. Hence, protein extraction method is suggested in order to isolate and purified protein in large amount.

Alkaline hydrolysis is a simple natural process where complex molecules are broken down into their constituent building blocks by the insertion of water (Gordon *et al.,* 2004). On the previous years, alkaline hydrolysis has been used to study chemical structure of biological molecules, make soaps from animal fats and to prepare skeletal remains for study. Alkaline hydrolysis is acted as improved alternative to incineration for disposing of waste biologic tissues and animal carcasses based on the same chemical reaction, with strong alkali and heat acts in increased speed the process (Thacker, 2004). This method usually used water solutions of alkali metal hydroxides such as sodium hydroxide (NaOH) or potassium hydroxide (KOH). The advantages of alkaline hydrolysis included combination of sterilization and digestion into one operation, reduction of waste volume and weight by as much as 97% complete destruction of pathogens including prions, production of limited odour or public nuisances, and elimination of radioactively contaminated tissues. In addition, heating the reactants dramatically accelerates hydrolysis.

In this study, EBN from *Collocalia Fuciphaga* is used as the raw material. The entire length and weight measured of *C.fuciphaga* is about 12cm and 15-18gm respectively. Besides, in many years, mitogen and avian epithelial growth factor that is known as hormone like substances is found in the *C.fuciphaga* (Jie *et al.*, 2009). EBN has been used for health and tonic as it often offers a good effect on curing tuberculosis, dry coughs, suppressing coughs and phlegm-dyspnea (breathing difficulty), treating consumptive diseases, alleviating hemoptysis (blood's cough), asthma, improving the voice, relieving gastric troubles, stomach ulcer, asthenia and main or common weakness of bronchial ailments. In traditionally, EBN also used to nourish the lungs heart, kidneys and stomach to aid renal functions, promoting growth, strengthen and enhance the immune system, improve concentration, regulate circulation and increase energy and metabolism of body (Kong *et al.*, 1987 and Chan, 2006).

Malaysia is currently one of the largest producers of EBN in the world and it publication can be hypothesized based on the enduring qualitative reasons below that:

1. Consumption of EBN is considered as a status symbol
2. The health giving properties of consuming EBN
3. Strong economic growth rates experienced by Hong Kong, China and Taiwan
4. Potential of EBN as a base mineral to be used in the production of herbal and vitamin supplement, the international market for EBN will grow at double-digit rates for the next two decades or so on.

1.2 Motivation

Every property that characterizes a living organism is affected by proteins. There are many function of protein that made it useful and needed in great amount so that the nutrients from the food can be obtained by all people who in need. Some of the function of protein are to store and transport a variety of particles ranging from macromolecules to electron as they guide the flow of electrons in the vital process of photosynthesis. Protein also acts as hormone, they transmit information between specific cells and organs in complex organism and in between proteins control the passage of molecules across the membranes that compartmentalize cells and organelles. Others than that, protein function in the immune systems of complex organisms to defend against intruders and control gene expression by binding to specific sequences of nucleic acids, thereby turning genes on and off (Lauritzen, 1992). One of the most crucial problems of the world is deficiency of food especially, in protein. About 500 million people suffer from severe-protein-calorie malnutrition. Furthermore, the increase of human population has caused serious problem (Lasztity *et al*., 1983). In order to obtain protein in great amounts, people may extract protein from any resources including marine life and porcine sources which sometimes do not suit to be consumed in large amounts by certain people. This is because, protein from marine life and other animal may contains high fat that will attributed heart diseases and blood pressure (Hoffman *et al.*, 2004), while protein from porcine sources are not Halal so this protein cannot be consumed by Muslim because of religious barrier. By then, in order to compete with protein extracted from animal and marine life, the percentage of protein extracted from EBN should be optimized. In addition, as there are many disadvantages of protein extraction from others sources, so, this study will focus on the extraction of protein from EBN via alkaline hydrolysis. In this study, the optimum condition for the protein extraction from EBN that are not been done before was being investigated.

1.3 Statement of problem

In order to produce the high yield of protein, the optimum condition for EBN extraction is determined instead of protein extraction of other sources. Previously, EBN has been extracted using water and enzymatic method which resulted 0.354 g/L (Suzana, 2012) and 0.493 g/L (Afifi, 2013) protein extract respectively. Thus, in this study the optimum condition of protein extracted from EBN using alkaline method will be determined by manipulating the extraction time, sodium hydroxide concentration and solid liquid ratio as there was lacking of research on the alkaline hydrolysis of EBN.

1.4 Objectives

The main objectives of this research are to extract protein from EBN using alkaline hydrolysis. This research also consists of a few specific objectives which are:

- To determine the effect of liquid solid ratio (LSR), extraction time and sodium hydroxide (NaOH) concentration on protein extract.
- To determine the optimum condition of protein extract during alkaline hydrolysis of EBN.

1.5 Scope of this research

- This research will be focusing on analyze EBN using UV-Vis Spectrophotometer
- This research also focus on characterization of EBN using Fourier Transform Infrared Spectroscopy (FTIR) and Inductively Coupled Plasma – Mass Spectroscopy (ICP-MS)
- Study the effects of three parameters, LSR (30:1 to 70:`1), reaction time (5 minutes to 25 minutes) and sodium hydroxide (NaOH) concentration on extraction protein concentration (40g/L to 120g/L)
- Response Surface Methodology (RSM) is used to determine the optimal extraction condition for the protein extraction using alkaline hydrolysis

1.6 Rationale and significance

Analysis of EBN chemical constituents can be traced back to 1921 (Wang, 1922). It is known that EBN is mainly composed of proteins. EBN has the properties of carbohydrate besides protein. As protein are the highest content in the EBN and it has valuable function to people, hence, the study of the extraction protein from EBN with the optimum condition is conducted so that the nutrients on the protein in the EBN is preserved as well as produced high amount of protein extract. However, it is not known yet which method of extraction protein will yield the large amount of total protein. So, this research is revealed that alkaline hydrolysis produced the highest protein yield compared to the acid and enzymatic hydrolysis.

2 LITERATURE REVIEW

2.1 Edible bird's nest (Collocalia Fuciphaga)

Around world, there are more than 24 species of ecolocating and insectivorous swiflets being distributed, but only a few produce nests that are deemed as 'edible' (Koon, 2000). Marcone (2005) stated that 'Edible bird's nest (EBN)' refers to the nest produced by several different swiftlet species which are *C. fuciphaga*, *C. germanis*, *C. maxima* and *C. unicolor* (Goh *et al*., 2001). This research is focused to *C. fuciphaga species* (Figure 2.1) which only be found in the Southeast Asian region. The consumption of these nests by human has been a symbol of wealth, power, and prestige, as well as being used medicinally in traditional Chinese medicine dating as far back as the Tang and Sung dynasties (Koon and Cranbrook, 2002).

Figure 2-1: *Collocalia Fuciphaga* swiflet

2.2 Types of edible bird's nest (EBN)

Nowadays, classification of EBN is usually based on the original place they are built (Goh *et al*., 2001). There are two main types of EBN which are House nest (Figure 2.2) and Cave nest (Figure 2.3). The House nest refers to nests that are built in wooden-roofed swallow farmhouses which are humid and dark. The interior temperature (28°C - 30°C), brightness and relative humidity (90%) are relatively suits to the bird's natural cave habitat. The swiftlet leaves the farmhouse to find food in the wild in the morning and returns in the evening. House nest consists of extremely less impurities and feathers since it farmhouse provides a better habitat. One of the characteristic of house nest is cleaner. House nest looks like a spoon and have a high swelling capacity. Besides, the House nest consists of three different colors which are white, yellow and bloody-red nests. The white nests are smooth contrast to yellow and bloody-red nests that are crunchier due to existences of high amount of minerals. The nest that was constructed in caves and on cliffs is known as cave nest. It is firm, hard and deep in color due to owing to the harsh natural climate and environment. It is not as well shaped as the house nest and exhibits higher impurities. The cave nest is crunchy with low abilities to swell that extends the time for it to swell when preparing, (Sam *et al*., 1991).

Figure 2-2: House nest of Edible bird's nest (EBN)

Figure 2-3: Cave nest of Edible bird's nest (EBN)

2.3 Nutritional value of edible bird's nest (EBN)

Due to the highly evaluated function both nutritiously (water-soluble protein, carbohydrate, iron, inorganic salt and fibre) and medically (anti-aging, anti-cancer, immunity-enhancing, etc), EBN has been esteemed a precious food tonic by Chinese people ever since the Tang dynasty and it was referred as "Caviar of the East". There is current scientific study confirmed that EBN has haemagglutination inhibiting activity against the influenza virus (Howe, 1961; Howe, Lee, and Rose, 1960). An update discovery demonstrated that partially purified swiftlet nest extracts possess the first known avian epidermal growth factor (EGF) (Kong *et al*., 1987; Ng, Chan, and Kong, 1986). Nowadays, EBN was developed into various food products including drink, food additive, and even cosmetic ingredient with the help of modern commercialization and technology. It was also found that both nests share a common 77 KDa protein that has properties similar to those of the ovotransferrin protein in eggs. This protein may responsible for the severe allergic reactions that sometimes occur among young children who consume EBN products (Marcone, 2005). EBN is not the only commercially available food product highly esteemed or priced for human consumption processed through an animal but also includes argan oil made from the argan nut that has passed through the digestive track of a goat and Kopi Luwak which is the most expensive and rarest beverage/coffee. More scientific work should be done in the future to fully elucidate the biological and medicinal functions of the EBN.

2.4 Origin of edible bird's nest (EBN)

The nests are built mainly by male swiftlet. It is made almost entirely from the saliva secreted by the swiftlet's two sublingual glands. The swiftlet's sublingual salivary glands increase their weight from 2.5 mg to 160 mg and reach maximum secretory activity during nesting and breeding season (Medway, 1962). The birds use saliva to bind materials together and attach them to the vertical walls of inland or seaside caves in order to construct their nests, (Kang *et al*., 1991). The weight of the nest can be 1–2 times the body weight of the swiftlets and the nests can only support the mother and the nestlings. Besides, the nest is looks like half-bowl (Figure 2.4) and 35 days may be needed for the construction process (Marcone, 2005.) The nests are built almost exclusively by the 7–20 g male swiflet over this period of time. Grade of EBN is determined by observing three parameters including dry mass, the duration of time the swiftlets spend in order to construct the nest, the fat and protein content of hardened saliva. Majority of EBN come from two exploited species which is White-nest swiflet and the Black-nest swiflet, whose habitats range from the Nicobar Islands in the Indian Ocean to sea-aves in the coastal regions of Thailand, Vietnam, Indonesia, Borneo and the Palawan Islands in the Philippines (Koon, 2000; Koon and Cranbrook, 2002). The white nest is made almost entirely from saliva (Sims, 1961) while the black nest contains about 10% feathers which contribute 8% of the protein content in the nest (Kang *et al*., 1991).

Figure 2-4: Edible bird's nest (EBN)

2.5 *Contents of edible bird's nest (EBN)*

There are some properties of proteins, peptides, amino acids, and nitrogen in the EBN as for example, the composition of nitrogen consists in the EBN is about 10.29% (Chen *et al*.,1996 ; Wang, 1921). For two types of nests, the white nest and the red blood nest, there is compositional properties of lipid (0.14 – 1.28%), ash (2.1%), carbohydrate (25.62 - 27.26%) and protein (62 – 63%)(Marcone, 2005). It has confirmed that EBN contains a higher percentage of humin nitrogen and cysteine nitrogen than for pure proteins which may be because of the carbohydrate radical and fine feathers present in the nest (Wang, 1921). It was known that EBN rich in protein which are composed of 20 amino acids. EBN contains all the essential amino acids as the most abundant amino acids in the EBN are serine, threonine, aspartic acid, glutamic acid, proline, and valine (Kathan and Weeks, 1969). While, the white nest, it is rich in two aromatic amino acids such as phenylalanine and tyrosine (Marcone, 2005).

2.6 Market of edible bird’s nest (EBN)

Hong Kong is world’s largest importer and consumer of the processed nests, while North America being the second largest market (Goh *et al*., 2001). 17–20 million nests were estimated are harvested each year with the total weight approximately about 2 metric tonnes (Goh *et al*., 2001). Depending upon grade, the EBN retails for anywhere from $2000.00 (for white nests) to $10,000.00 (for “red blood” nests) Canadian per kilogram (Koon and Cranbrook, 2002)

2.7 Pre-treatment of edible bird’s nest (EBN)

EBN need to be cleaned right after the collection and the procedure is known as pre-treatment. It is a long and time-consuming process. First of all, the raw EBN is soaked in water for 6 to 48 h for them to absorb the water and expand. Then, the removal of the feathers is performed in which the larger feathers are removed by using tweezers while fine feathers are removed with a floatation technique using vegetable oil as this procedure is depends on the processors. In order to produce premium grade nests, the long strands of nest cement are carefully been isolated. Instead of that, the broken strands are meshed and used to constitute the base of the cleaned nests. The color of processed nests resembles that of the raw nests. Besides that, the processors do not normally clean the good quality white nests because it's difficult to restore them to their original shape after they have been soaked in water and for the information, the process may take a person 8 h to clean about 10 nests (Koon and Cranbrook, 2002; Leh, 2001).

2.8 Swiflet farming in Malaysia

There are fundamental and long-standing industries in Malaysia such as rubber, palm oil, oil and gas, timber and financial services, but since 5 years ago, swiflet farming industry being new development in this country. Such good news, due to the industry’s relatively profitable risk-return profile followed by a continuously growing demand for EBN by wealthy overseas countries, the swiftlet farming industry has potential to grow into a multi-million ringgit industry. In order to produce natural and organic health supplement products for local and overseas consumption, there is also a discernable world-wide trend pursued by international as well as home grown pharmaceutical and herbal products companies using EBN as base materials. As the swiftlet farming industry continues to expand and grow, more and more supplies of EBN sourced from purpose-built farms that are constructed specially to house EBN swiftlets find their way into the supply chain (Hameed, 2007).

After the Asian Economic Crisis (1997-1998), the swiftlet farming industry in Malaysia has began to gather momentum. At this time, many businesses, excluding larger business experienced hard times and a great number of them closed down throughout the country. Over the last 8 years, the swiftlet farming industry in Malaysia has been growing and before 1998, it has been estimated that there are 900 plus swiftlet farms throughout the country. There are number of businessmen, landlords and investors has began to realize that the financial viability of the swiftlet farming industry in Malaysia right after the first generation of swiftlet farms that are established after 1998 started to produce commercially harvestable quantities of EBN. By the end of 2006, the estimation of swiftlet farms throughout the country close to 36,000 units, with an average annualized growth rate of 35% per year (Hameed, 2007).

Nowadays, the business of swiftlet farming essentially involves the conversion of people-centric buildings into buildings used to house and protect a certain species of swiftlets that can be found in the South East Asian region as well as the design and construction of purpose-build buildings for the purposes of accommodating such swiftlet populations as well. Each and every day, a continuous vocalization of swiftlet chirps and mating sounds are played using speakers and audio systems installed within such buildings in order to lure the swiftlets that are flying overhead to fly into the said buildings to mate and make the buildings their new home. There are almost 99% of all swiftlet farms in Malaysia are geared towards the production of white edible birds' nests (Hameed, 2007).

Mostly, secondary and tertiary townships is often being a choice of major swiflet farming areas due to the abundance of food source is and pollution levels are at their relative minimum. These secondary and tertiary townships include Kampong Tebing, Kampong Tasoh, Kampong Banat Bawah, Jampong Bakan, Kuala Nerang, Pokok Sena, Kampong Tanjung Radin, Kuala Ketil, Lunas, Kulim, Sungai Petani, Jitra, Bukit Mertajam, Nibong Tebal, Kepala Batas, Cangkat Kledang, Legong, Jelai, Cangkat Jering, Bruas, Pantai Remis, Lumut, Teluk Intan, Setiawan, Bagan Serai, Parit Buntar, Selama, Tanjung Malim, Kuala Kubu Bahru, Rawang, Kepong, Cheras, Slim River, Kulai, Kanpong Bahru Paroi, Alor Gajah, Ayer Pasir, Durian Tunggal, Tangkok, Pagoh, Bukit Pasir, Kampong Machap, Ulu Tiram, Tai Hong Village, Senai, Pontian Kecil, Jemaluang, Kampong Seri Pantai, Mersing, Kampong Sawah Datuk, Kampong Air Papan, Kuala Besut, Tok Soboh, Kampong Pinang, Rompin, Pekan, Kuala Terengganu and Pasir Mas (Hameed, 2007). In this study, the raw material has been taken from Pekan, Pahang Darul Makmur.

Currently, behind Indonesia (60%) and Thailand (20%), the third largest producer of EBN (7% of gross supply value) in the world is comes from Malaysia. In 2006, about 90 to 120 of unprocessed white EBN is able to fetch production level prices of RM$4500 to RM$6000. A kilogram of processed white EBN is able to fetch retail level prices of RM$15000 to RM$25000 in Hong Kong and China (Hameed, 2007).

Hong Kong (50% of world trade), China (8%), Taiwan (4%) and Macau (3%) has been categories as the main export markets for EBN with a consumption weight value of approximately 160 tons for 2006. The total consumption value of EBN was estimated to be in the range of RM8 billion to RM12 billion. In 2004, Ministry of Housing and Local Government with the gazzetting of the "Guidelines on Swiftlet Farming" recognized the swiftlet farming industry in Malaysia as a valid contributor of important foreign exchange currency for the country (Hameed, 2007).

The Guidelines require registered premises with relevant council that have not been designated as a Class 1 Heritage Building converted into a swiftlet farm. The Guidelines also require that certain standards and levels of premises upkeep must be adhered to in areas of noise, health, pollution, scope of renovation works, building façade rendition and lighting be adhered to before swiftlet farming licenses can be issued by the relevant council (Hameed, 2007).

A swiftlet farm can yield returns that are competitive with other types of profitable industries if it been constructed properly. There are many swiftlet farms throughout the country yield 1kg to 10kgs (RM4000 to RM40000) of collected white EBN per month based on perceptive and cognitive observations. On average, an average yield of around 1kgs to 3kgs (i.e. RM4000 to RM12000) of collected white EBN per month can be obtained from a 2 storey swiftlet farm that had been properly converted from a conventional shop-lot or shop-house in Malaysia (Hameed, 2007).

2.9 Protein

The bioactive components contains in the EBN (protein) need to be isolated and purified as it consists of many functions that benefits human being. Protein acts as an essential nutrient for human body which is not only as an energy source but also as a building agent. Protein is used to build and maintain body tissues, produce neurotransmitter for brain, produce amino acids and hormones, maintain immunity system and also maintain acid-base balance in cell fluid. Apart from that, protein also functioned well in body growth. In addition, protein also plays important roles in phenomena such as signal transduction, gene expression, metabolism, cellular and extracellular structures, etc. Denise *et al*., (2001) revealed that promotion of cell division and epidermal growth-factor like proteins are capable in the presence of protein. Many proteins are also useful for therapeutic or diagnostic applications. Besides, any property on which the utility of food proteins depends on the food ingredients (Cepeda *et al*., 1998). Many reasons food proteins are hydrolyzed such as for the improvement of nutritional and functional properties, texture characteristics to the removal of odour, flavour, and toxic or anti-nutritive components. In hydrolysis treatments, general proteins had used are casein, whey and soy protein (Periago *et al*., 1998). Therefore, it is still necessary to prepare the protein of interest in a pure form without contaminants that may potentially put a human being's health at risk. Separation and purification of proteins constitute a major bottleneck of modern process biotechnology.

2.10 Source of protein

Commercially available protein foods are obtained from a range of animal and plant sources and are used as functional ingredients (Periago *et al*., 1998). Due to the increasing costs and limited supplies of animal proteins, and since vegetable protein is the most abundant source of protein on the Earth, a number of vegetable proteins have been investigated for possible incorporation into formulated foods (Achouri *et al*., 1999; Sathe and Sze-Tao 2000). The examples of vegetables protein are alfalfa leaf, cottonseed, winged bean, peanut and soya.